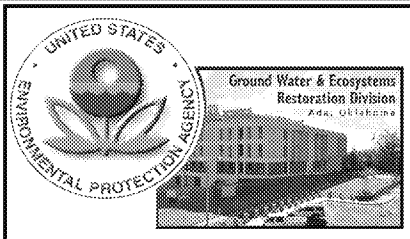


**U.S. EPA OFFICE OF RESEARCH & DEVELOPMENT**

**GROUND WATER & ECOSYSTEMS RESTORATION DIVISION**

# SEMINAR SERIES

## Establishing and Optimizing a UHPLC-MS-SPE-NMR System for the Identification of Plant Metabolites

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**Wednesday, January 27, 2016**
**10:00 AM - Presentation**
**RSKERC Conference Room 1**

### Abstract

Introduction: Large-scale profiling of plant metabolites helps to advance our fundamental understanding of plant biochemistry and yields discovery of novel metabolites and gene functions. However, the depth-of-coverage of identified plant metabolites is estimated to be between 10% and 20%. We have addressed this limitation by developing a UHPLC-QToFMS-SPE-NMR platform which facilitates and accelerates the systematic and biologically motivated identification of the metabolites in the model legume, Medicago truncatula. This platform has been used to confirm the chemical structure of over thirty metabolites in Medicago truncatula, some of which have not been reported before. As a result, the biological context of our metabolomics experiments are increasing, thus providing even greater opportunities and understanding of this model legume.

Methods: Systematic and biologically driven metabolite identifications were pursued using a UHPLC-ESI-QToF-MS-SPE-NMR platform. For this study, aerial and root tissues of Medicago truncatula were extracted and analyzed. An efficient, automated UHPLC-ESI-QToF-SPE method was developed for the isolation and concentration of targeted compounds, thereby, enabling higher-throughput structural identification by NMR analyses. Accurate mass measurements, isotopic ratios, elemental formulas, and MS/MS spectra from UHPLC-ESI-QToF-MS are being used along with in silico software (PlantMAT) to provide putative identifications of metabolites. Targeted UHPLC chromatographic peaks were isolated, trapped and concentrated using automated SPE. Aided with the putative identifications provided by PlantMAT, full compound structural confirmation of the isolated compounds was done by 1D and 2D NMR analysis.

Preliminary Results: Although routine LC/MS analysis of aerial/root tissue from Medicago truncatula can be accomplished using 10 mg of dried tissue, a mass of 100-1000 mg was needed to provide sufficient isolated/purified compound (>1μg) for NMR analysis. For automated SPE, it was determined that Waters Oasis HLB (hydrophilic/lipophilic balance) cartridges (1 mm x 10 mm) yielded the best recoveries. The HLB cartridges were eluted with 250 μL of methanol-d3. The entire eluent from the SPE cartridge was collected in autosampler vials and the solvent evaporated to a volume of 30 μL before transfer to 1.7 mm o.d. NMR tubes. The average solid phase extraction recovery of this method was determined to be 93% for nine flavonoids and saponins. The work-flow for compound identification in plant tissue extracts consists first of dereplication of those chromatographic peaks that can be identified through mass spectra matching with authentic standards present in our MS and MS/MS libraries, putative, and NMR identified compounds. Then, unidentified chromatographic peaks were targeted based upon their biological significance. As many as twenty-five replicate injections of the tissue extract were concentrated on individual cartridges for each targeted peak. A UHPLC mass chromatogram of an extract of aerial Medicago truncatula tissue will be provided with peaks annotated with the NMR confirmed structures including over thirty polyphenolic glycosides, saponins, phospholipids, and fatty acids.